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Applicant: REYES, Antonio et al. Conf.: 1014
Appl. No.: 09/544,773 Group: 1656
Filed: April 7, 2000 Examiner: MEYERS, C.
For: LINKED LINEAR AMPLIFICATION OF NUCLEIC
ACIDS

AMENDMENT

RECEIVED

Assistant Commissioner for Patents
Washington, DC 20231

APR 19 2002

April 3, 2002

TECH CENTER 1600/2900

Sir:

In reply to the Examiner's Office Action dated October 3, 2001, the period for response have been extended by three (3) months to April 3, 2002, the following amendments and remarks are respectfully submitted in connection with the above-identified application.

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IN THE SPECIFICATION

Please amend the Specification as follows:

Please replace the paragraph beginning on page 12, line 21 with the following paragraph:

61 Figure 12 is a schematic representation of the sequence of the human β -globin gene (GenBank locus HUMHBB; SEQ ID NO.:1) and of several primers described herein.

Please replace the paragraph beginning on page 13, line 7 with the following paragraph:

62 Figure 13 is the sequence of Human Growth Hormone Sequence (SEQ ID NO.: 15) Example 7. The relative positions of the oligonucleotides used in the experiment are underlined. Oligonucleotide GH1 hybridizes to the complement of the Human Growth Hormone sequence shown.

Please replace the paragraph beginning on page 34, line 1 with the following paragraph:

63 A template with or without a single nucleotide base containing a 1,3 propane diol moiety (designated as non-replicable element "X") and a primer complementary to the 3' end of the template were synthesized in order to demonstrate the ability of the 1,3 propane diol moiety to serve as a non-replicable element and halt DNA synthesis. The sequences synthesized are as follows:

<u>Name</u>	<u>Sequence (5' -> 3')</u>	<u>SEQ ID NO.</u>
DNA II 207	GCTCCCTTAGCATGGGAGAGTCTCCGGTTC	1
DNA II 207X	GCTCCCTTAXCATGGGAGAGTCTCCGGTTC	2
P12	GAACCGGAGACT	3

The primers and templates were annealed to form the following primer template complexes:

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5' GCTCCCTTAGCATGGGAGAGTCTCCGGTTC
TCAGAGGCCAAG5'

SEQ ID NO.:1
SEQ ID NO.:3

5' GCTCCCTTAXCATGGGAGAGTCTCCGGTTC
TCAGAGGCCAAG5'

SEQ ID NO.:2
SEQ ID NO.:3

The primer template complexes were then extended with various DNA polymerase (Perkin Elmer - Cetus Corp.), BST polymerase I, AmpliTaq polymerase (Bio-Rad Laboratories, Hercules, CA) and Sequenase polymerase (United States Biochemical, Cleveland, OH) in the presence of α -[32P]-dCTP and the products were subjected to electrophoresis on a denaturing polyacrylamide gel.

Please replace the paragraph beginning on page 35, line 17 with the following paragraph:

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Four oligonucleotides were prepared, two of which contained the non-replicable 1,3-propane diol moiety. The sequences were synthesized on an Eppendorf Ecosyn D300, automated DNA synthesizer with the MMT-propane diol phosphoramidite in the Z position. When entering the sequence into the synthesizer, a Z was introduced. The sequences are presented in the following table:

Name	Sequence (5' -> 3')	SEQ ID NO.:
BGP-2 22	GGGTGGGAAAATAGACCAATG	4
BGP-2 22X	GGGTGGGAAAATAGACCXATG	5
BGP-1 30	GGCAGGAGCCAGGGCTGGGCATAAAAGTCA	6
BGP-1 30X	GGCAGGAGCCAGGGCTGGGCATAAAXGTCA	7

Please replace the paragraph beginning on page 36, line 4 with the following paragraph:

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The sites of complementarity of the oligonucleotides in the human β -globin gene (GenBank Locus HUMHBB; SEQ ID NO.:1) is shown in Figure 12.

Please replace the paragraph beginning on page 38, line 8 with the following paragraph:

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The products of the reactions (3 μ l) were mixed with 10 μ l 4N NaOH, 250 mM EDTA and were blotted onto a Zeta-Probe membrane (Bio-Rad Laboratories, Hercules, CA). Also included on the membrane was 56, 118 and 231 ng of the plasmid pHB^A similarly denatured. The membrane was hybridized with 5'-³²P-CTGCAGTAACGGCAGACTTCTCCT (SEQ ID NO.:8) at 55°C for three hours in 5X SSPE, 1% SDS, 5 mg/ml Blotto, 10 μ g/ml Homomix I RNA. After hybridization the blot was washed at room temperature in 6X SSC and then scanned in a Bio-Rad Molecular Imager. The reaction produced approximately 250 fold amplification demonstrating the process of the present invention results in the amplification of the nucleic acid sequence of interest.

Please replace the paragraph beginning on page 39, line 15 with the following paragraph:

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To control the size of the fragments generated by the amplifications, two reactions were performed. One reaction contained Thermus aquaticus polymerase buffer, 5 pmol of primers BGP5-22X and BGP4-22X, 1×10^8 molecules of plasmid pHB^A, and 3 units of Ampli-Taq Polymerase. The second control included the same ingredients as the previous reaction except that the

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primers BGP1-35X and BGP2-35X were used. The template DNA was denatured for 4 min at 94°C and then cycled 48 times using the following conditions program: annealing and polymerization at 55°C for 30 sec; denaturation at 94°C for 30 sec. At the end of the last cycle the samples were annealed at 55°C for 30 sec and polymerized at 72°C for 4 min.

<u>Primer</u>	<u>Sequence (5' → 3')</u>	<u>SEQ ID NO.:</u>
BGP1-35X	CCAGGGCTGGGCATAAAAGTCAGGGCAGAG X CATC	9
BGP2-35X	GGGTGGGAAAATAGACCAATAGGCAGAGAG X GTCA	10
BGP4-22X	CCAAAGGACTCAAAGAA X CTCT	11
BGP5-22X	CCTCACCCCTGTGGAGCC X CACC	12

Please replace the paragraph beginning on page 40, line 8 with the following paragraph:

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The entire reaction (15 µl) was mixed with 1.6 µl 10X Ficoll loading buffer and subjected to electrophoresis in a 1.5% agarose gel (Bio-Rad ultrapure agarose). Electrophoresis was performed in TBE buffer for 90 min at 110 volts. The gel was subsequently stained with ethidium bromide (1 µg/ml) for 30 min, destained for 15 min, and photographed by ultraviolet (UV) illumination. The electrophoresed DNA was then transferred to a

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cor'dd nylon membrane (Zeta probe, Bio-Rad) by alkaline transfer (Reed, K. C. and D. A. Mann, Rapid transfer of DNA from agarose gels to nylon membranes, Nucleic Acids Res. 13:7207-7221 (1985)) and fixed to the membrane by UV radiation (Church, G. M. and W. Gilbert, Genomic Sequencing, Proc. Natl. Acad. Sci. U. S. A. 81:1991-1995 (1984)). The membrane was prehybridized in 5X SSPE, 1% SDS, 10 µg/ml homomix RNA and 0.5% dehydrated powdered skim milk (Carnation, Los Angeles, CA) for one hour and subsequently hybridized with 2.5×10^6 cpm/ml 5' end ^{32}P labeled probe 5'CAGGAGTCAGGTGCACCATGGT (SEQ ID NO.:13) for two hours at 55°C. The membrane was washed twice with 6X SSC for 30 min at room temperature and autoradiographed at room temperature for 30 min.

Please replace the paragraph beginning on page 42, line 4 with the following paragraph:

89 Two fold serial dilutions of the LLA and PCR products (1/64 µl, 1/32 µl, 1/16 µl, 1/8 µl, 1/4 µl, 1/2 µl, 1 µl, 2 µl, 4 µl, and 8 µl) were mixed with 1x Ficoll loading buffer and subjected to electrophoresis in a 1.5% agarose gel. Electrophoresis was performed in 1x TBE buffer for 90 min at 110 volts. The electrophoresed DNA was transferred to a nylon membrane by alkaline transfer (Reed and Mann, 1985), cross

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linked by UV radiation (Church and Gilbert, 1984), and then neutralized with 2x SSC. Subsequently, the membrane was hybridized in 5x SSPE, 1% sodium dodecyl sulfate (SDS), 10 µg/ml homomix RNA, 0.5% powdered skim milk and 2.5×10^6 cpm of the ^{32}P labeled probe 5' CAGGAGTCAGGTGCACCATGGT (SEQ ID NO.:13). The hybridization took place at 55°C for two hours. After hybridization, the membrane was washed twice with 6x SSC at room temperature for 15 min. and then scanned and quantified with the Bio- Rad GS-250 Molecular Imager.

Please replace the paragraph beginning on page 43, line 11 with the following paragraph:

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The 15 µl reaction volumes were mixed with 1.6 µl 10X Ficoll loading buffer and subjected to electrophoresis in a 1.5% agarose gel (Bio-Rad ultrapure agarose). Electrophoresis was performed in 1x TBE buffer for 90 min at 110 volts. The gel was subsequently stained with ethidium bromide (1 µg/ml) for 30 min, destained for 15 min, and photographed by ultraviolet (UV) illumination. The electrophoresed DNA was then transferred to a nylon membrane by alkaline transfer (Reed and Mann (1985)) and fixed to the membrane by UV radiation (Church (1984)). The membrane was prehybridized in 5X SSPE, 1% SDS, 10 µg/ml Homomix

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RNA and 0.5% dehydrated powdered skim milk for one hour and subsequently hybridized with 2.5×10^6 cpm/ml of the 5'-P³² labeled probe 5' CAGGAGTCAGGTGCACCATGGT (SEQ ID NO.:13) for two hours at 55°C. The membrane was washed twice with 6X SSC for 30 min at room temperature and the reactions were quantified with the Bio-Rad GS-250 Molecular Imager.

Please replace the paragraph beginning on page 44, line 16 with the following paragraph:

BII Oligonucleotides and ribonucleoside containing

oligonucleotides of the following sequences were prepared using standard automated solid-phase synthesis methods. The uracil nucleoside in oligonucleotides GH1 and GH2 are ribonucleosides.

<u>Name</u>	<u>Sequence (5' → 3')</u>	<u>SEQ ID NO.:</u>
GH1 SEQ	TTCCCAACCAUTCCCTTA	14
GH2 SEQ	GGATTCTCTGUTGTGTTTC	15
GH3 SEQ	TTCCCAACCATTCCTTA	16
GH4 SEQ	GGATTCTGTGTGTTTC	17
Md114	TAGCGTTGTCAAAAAGCC	18

Please replace the paragraph beginning on page 47, line 19 with the following paragraph:

612 LLA primers containing the non-replicable propanediol element ("X") were synthesized. Primers flank the β^s (20A>T) and β^c (19G>A) mutation of the human β -globin gene (see Figure 14). Primer sequences (5' -> 3') are as follows:

UP 8	TAAGCCAGTGCCAGAAGAGCCAAXGAC	SEQ ID NO.:19
UP 7	TACGGCTGTCATCACTTAGACXTCA	SEQ ID NO.:20
UP 6	CCCTGTGGAGCCACACCCTAGXGTT	SEQ ID NO.:21
UP 5	AATCTACTCCCAGGAGCAGGGXGGG	SEQ ID NO.:22
UP 4	GAGCCAGGGCTGGGCATAAAAXTCA	SEQ ID NO.:23
UP 3	GGCAGAGCCATCTATTGCTTAXATT	SEQ ID NO.:24
UP 2	TGCTTCTGACACAACCTGTGTTXACT	SEQ ID NO.:25
UP 1	AGCAACCTCAAACAGACACCAXGGT	SEQ ID NO.:26
LP 2	CCTCACCACCAACTTCATCCAXGTT	SEQ ID NO.:27
LP 3	AACCTTGATACCAACCTGCCCXGGG	SEQ ID NO.:28
LP 4	TATTGGTCTCCTTAAACCTGTXTTG	SEQ ID NO.:29
LP 5	TTCTCTGTCTCCACATGCCAXTTT	SEQ ID NO.:30
LP 6	CAGTGCCTATCAGAAACCCAAXGAG	SEQ ID NO.:31
LP 7	AAATAGACCAATAGGCAGAGAXAGT	SEQ ID NO.:32
LP 8	GACCACCAGCAGCCTAAGGGTXGGA	SEQ ID NO.:33
LP 9	TTCCTATGACATGAACTTAACXATA	SEQ ID NO.:34
LP 10	AAACTGTACCCTGTACTTCTXCCC	SEQ ID NO.:35
LP 11	CAATCATTCGTCTGTTTCCCAXTCT	SEQ ID NO.:36

Please replace the paragraph beginning on page 49, line 5 with the following paragraph:

613 To compare LLA and PCR efficiencies, PCRs were performed using

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the following primers (5' -> 3') corresponding to UP 8, LP 8 and LP 11 but without the non-replicable propanediol element "X":

UP 8-x	TAAGCCAGTGCCAGAAGAGCCAAGGAC	SEQ ID NO.: 37
LP 8-x	GACCACCAGCAGCCTAAGGGTGGGA	SEQ ID NO.: 38
LP 11-x	CAATCATTCGTCTGTTTCCCATTCT	SEQ ID NO.: 39

Please replace the paragraph beginning on page 49, line 18 with the following paragraph:

B14
PCR and LLA amplification products were labeled in a primer extension reaction using the 5'-biotinylated primer MD 792 (5' CACCTTGCCCCACAGGGCAGTAACG 3' (SEQ ID NO.:84); see Figure 14). A 2 µl-aliquot of the LLA or PCR reaction was mixed with 18 µl of a mixture containing 10 pmoles MD 792, 10 mM Tris-HCl pH 9.2, 50 mM KCl, 2.5 mM MgCl₂, 0.2 mM dNTP's and 1 U AmpliTaq DNA polymerase. Reactions were placed in the thermal cycler and subjected to one cycle of heating and cooling as follows: 94°C for 2 minutes, 55°C for 2 minutes, 72°C for 5 minutes.

Please replace the paragraph beginning on page 53, line 1 with the following paragraph:

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Eighteen Factor V gene specific primers, each containing the non-replicable propanediol element near the 3'-end, were synthesized. The primers flank the Factor V Leiden mutation (1691 G>A) site in exon 10. The 5' to 3' sequences are shown below:

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FVU9	AGCACACCAACATGACACATGXATA	SEQ ID NO.:40
FVU8	CACATGTACCCTAGAACTTAAAGXATA	SEQ ID NO.:41
FVU7	AATTGGTTCCAGCGAAAGCXTAT	SEQ ID NO.:42
FVU6	CAGGCAGGAACAACACCATXATC	SEQ ID NO.:43
FVU5	AGAGCAGTTCAACCAGGGGXAAC	SEQ ID NO.:44
FVU4	TCTTAGAGTTTGATGAACCCAXAGA	SEQ ID NO.:45
FVU3	AAATGATGCCCAGTGCTTAACXAGA	SEQ ID NO.:46
FVU2	CCATACTACAGTGACGTGGACXTCA	SEQ ID NO.:47
FVU1	GAGAGACATCGCCTCTGGGXTAA	SEQ ID NO.:48
FVL1	TTCTAGCCAGAAGAAATTCTCXGAA	SEQ ID NO.:49
FVL2	TTATTTAGCCAGGAGACCTAAXATG	SEQ ID NO.:50
FVL3	CTGTTCTCTTGAAGGAAATGCXCCA	SEQ ID NO.:51
FVL4	GGTGCTAAAAAGGACTACTTGXCAA	SEQ ID NO.:52
FVL5	CTTCGGCAGTGATGGTACTGAXAAA	SEQ ID NO.:53
FVL6	AACAGACCTGGAATTTGAAACXAAG	SEQ ID NO.:54
FVL7	CAACTTGCTCAACACATCCAAXACC	SEQ ID NO.:55
FVL8	AAGGAAGAAATTAGGAAAGGCXAAT	SEQ ID NO.:56
FVL9	CATTTTtaggagggttattaccxatt	SEQ ID NO.:57

Please replace the paragraph beginning on page 54, line 8 with the following paragraph:

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To label the amplification products, a 4 µl-aliquot of the LLA reaction was mixed with 38 µl of a mixture containing 10 pmoles of 5'-biotinylated MD 792, 10 pmoles of 5'-biotinylated FVU0 (5' CTACTTCTAATCTGTAAGAGCAGA 3'; SEQ ID NO.:58), 10 pmoles of biotinylated FVL0 (5' GAAAGGTTACTTCAAGGACAAA 3'; SEQ ID NO.:59), 10 mM Tris-HCl pH 9.2, 50 mM KCl, 2.5 mM MgCl₂, 0.2 mM dNTP's and 2 U

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AmpliTaq DNA polymerase. Primer extension reactions were placed in the thermal cycler and subjected to one cycle of heating and cooling as follows: 94°C for 2 minutes, 60°C for 2 minutes, 72°C for 5 minutes.

Please replace the paragraph beginning on page 56, line 14 with the following paragraph:

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The post-amplification and concerted methods of labeling were compared in the detection of the Factor V Leiden mutation. In the case of post-amplification labeling, the LLA reaction contained 10 pmoles of each primer in the Factor V 18 primer set, template DNA, 0.2 mM dNTPs, 10 mM Tris-HCl pH 9.2, 50 mM KCl, 2.5 mM MgCl₂, and 2 U AmpliTaq DNA polymerase (Perkin-Elmer) in a total volume of 50 µl. Reactions were placed in a thermal cycler (Perkin-Elmer 9600), heated at 94°C for 2 minutes, then subjected to 35 cycles of heating and cooling as follows: 94°C, 45 seconds; 50°C, 30 seconds; 72°C, 30 seconds. Finally, the reactions were heated at 72°C for 5 minutes. Amplification products were labeled by primer extension using 10 pmoles of 5'-biotinylated FVU0.1 (5' CTAATCTGTAAGAGCAGATCCCXGGA 3'; SEQ ID NO.:60) and 10 pmoles of 5'-biotinylated FVL0.1 (5' TTCTGAAAGGTTACTTCAAGGAXAAA 3'; SEQ ID NO.: 61) as primers.

Please replace the paragraph beginning on page 59, line 3 with the following paragraph:

618 LLA primers containing the non-replicable propanediol element ("X") were synthesized. These primers correspond to the human β -globin cDNA sequence (Genbank Accession No. NM_000518). Primer sequences (5' -> 3') are as follows:

UP1	AGCAACCTCAAACAGACACCA XGGT	SEQ ID NO.:62
UP2.1	TTTGCTTCTGACACA ACTGTGTT CACXAGC	SEQ ID NO.:63
UP9	TACCCTTGGACCCAGAGGTT CXTTG	SEQ ID NO.:64
UP10	AGTCCTTTGGGGATCTGTCCXCTC	SEQ ID NO.:65
UP11	GCTGTTATGGGCAACCCTAAXGTG	SEQ ID NO.:66
UP12.1	AAGGCTCATGGCAAGAAAGXGCT	SEQ ID NO.:67
UP15	GCACCTGACTCCTGAGGAGAAXTCT	SEQ ID NO.:68
UP16	CCGTTACTGCCCTGTGGGXCAA	SEQ ID NO.:69
UP17	AACGTGGATGAAGTTGGTGGTXAGG	SEQ ID NO.:70
LP25	CACAGTGCAGCTCACTCAGTGXGGC	SEQ ID NO.:71
LP26	TCTCAGGATCCACGTGCAGCXTGT	SEQ ID NO.:72
LP27	AGACCAGCACGTTGCCCAXGAG	SEQ ID NO.:73
LP28	CCAAAGTGATGGGCCAGCXAC	SEQ ID NO.:74
LP29	GCACTGGTGGGGTGAATTCXTTG	SEQ ID NO.:75
LP30	GCCACCACTTTCTGATAGGCAXCCT	SEQ ID NO.:76
LP31	TGTGGGCCAGGGCATTAGXCAC	SEQ ID NO.:77

Please replace the paragraph beginning on page 60, line 2 with the following paragraph:

B19 First-strand cDNA was prepared by reverse

transcription using a propanediol-substituted oligonucleotide β RT1 primer (TGGACAGCAAGAAAGCGAGCXTAG; SEQ ID NO.:78) and the SuperscriptTM Preamplification System (Life Technologies). One (1) μ l of whole blood total RNA (approximately 1 μ g), 1 μ l of 10 μ M β RT1 primer and 10 μ l of water were mixed and heated at 70°C for 10 minutes, then at 55°C for 1 minute. After the addition of four μ l of 5X First Strand Buffer (1X buffer contains 50 mM Tris-HCl, pH 8.3, 75 mM KCl, 3 mM MgCl₂), 1 μ l of 10 mM dNTPs and 2 μ l of 0.1 M dithiothreitol, the sample was heated at 55°C for 1 minute. One (1) μ l of Superscript II reverse transcriptase (200 Units) was then added and the mixture incubated sequentially at 55°C for 30 minutes, 70°C for 15 minutes and 37°C for 1 minute. Finally, 0.5 μ l of RNase H (1 Unit) was added and the mixture heated at 37°C for 20 minutes. The final volume of the cDNA preparation was 20.5 μ l. As a control, a parallel reaction was carried out using a non-substituted oligonucleotide β RT1-x primer (TGGACAGCAAGAAAGCGAGCTTAG; SEQ ID NO.:79).

Please replace the paragraph beginning on page 60, line 16 with the following paragraph:

B20 In order to assess the efficiency of the priming reaction conducted with the propanediol-substituted oligonucleotide β RT1 primer, PCR reactions were carried out using an aliquot of each first-strand cDNA sample (primed with β RT1 or β RT1-x) and PCR primers UP2-x (TTTGCTTCTGACACAACTGTGTTTCAC; SEQ ID NO.:80) and LP26-x (TCTCAGGATCCACGTGCAGCTTGT; SEQ ID NO.:81). Each PCR reaction contained 10 pmoles of UP2-x; 10 pmoles of LP26-x; the equivalent of 0.08 μ l, 0.4 μ l or 2 μ l of first strand cDNA preparation; 0.2 mM dNTPs; 10 mM Tris-HCl pH 9.2; 50 mM KCl; 2,5 mM $MgCl_2$, and 1 U AmpliTaq DNA polymerase in a total volume of 25 μ l. Samples were placed in the thermal cycler, heated at 94°C for 2 minutes, then subjected to 17 cycles of heating and cooling as follows: 94°C, 45 seconds; 62°C, 15 seconds; 72°C, 30 seconds. At the end of the last cycle, reactions were heated at 72°C for 4.5 minutes.

Please replace the paragraph beginning on page 62, line 7 with the following paragraph:

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Amplification products were labeled in a primer extension reaction using the 5' biotinylated primer biotin-LP24 (AAAGGTGCCCTTGAGGTTGTCCAG; SEQ ID NO.:82). A 10 ul aliquot of the LLA or PCR reaction was mixed with 1 ul of 10 uM biotin-LP24. Samples were placed in the thermal cycler and subjected to one cycle of heating and cooling as follows: 94°C, 2 minutes; 55°C, 2 minutes; 72°C, 5 minutes.

Please replace the paragraph beginning on page 62, line 13 with the following paragraph:

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Biotin-labeled DNA fragments were detected colorimetrically after capture in microwells coated with oligonucleotide 010599 (CCTTTAGTGATGGCCTGGCT; SEQ ID NO.:83). Detection reagents used were components of the Bio-Rad mDx Variant Gene 1 kit. The primer extension-labeling reaction (11 ul) was mixed with 10 ul of Denaturation Solution then incubated for 10 minutes at room temperature. A 10 ul aliquot of the denatured sample and 40 ul of hybridization solution were loaded into a well containing immobilized oligonucleotide 010599. The well was incubated for 1 hour at 37°C then washed five times with Well Wash Buffer. Then, 50 ul of SA-HRP (streptavidin-horseradish peroxidase conjugate) were added to the well. The

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conclg well was incubated for 30 min. at 37°C then washed five times with Well Wash Buffer. Next, 50 ul of Substrate TMB (tetramethyl benzidine) were added to the well. After 10 minutes at room temperature, the colorimetric reaction was stopped by adding 50 ul of Stop Solution. Absorbance was read at 450 nm with 595 nm as reference wavelength.

IN THE CLAIMS

Please amend the claims as follows:

B23 1. (Amended) A process for generating a complementary DNA (cDNA) molecule from an RNA molecule comprising:

- a) annealing a first primer containing a non-replicable element, with or without a cleavable element, to an RNA molecule,
 - b) generating a first strand cDNA product,
 - c) annealing a second primer containing a non-replicable element, with or without a cleavable element, to the first strand cDNA product, and
 - d) generating a second strand cDNA product that is a complement of said first cDNA strand.
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2. A process for amplifying a cDNA molecule comprising:

- a) generating a cDNA molecule according to the process of claim 1 ;
- b) combining said first and said complement cDNA strands in a reaction mixture with a primer containing a non-replicable element and/or a cleavable element, under conditions such that first generation primer extension products are produced using said strands as templates, and wherein said primer for a cDNA strand is selected such that a first generation primer extension product produced using said cDNA strand as a template, when separated from said cDNA strand, can serve as a template for synthesis of a second generation primer extension product of the primer for said complement strand;
- c) separating said first generation primer extension products; from their respective templates to produce single-stranded molecules; and
- d) treating the first generation primer extension products with the primers of claim 2, step (b) under conditions such that second generation primer extension products are produced using the first

generation primer extension products as templates, wherein the second-generation primer extension products contain at least a portion of the sequence of the nucleic acid sequence of said first generation primer extension product and no more than an insufficient portion of the binding site for said first primers for producing said first generation primer extension products of claim 2.

B24 3. (Amended) A process for amplifying a cDNA molecule comprising:

- (a) generating a cDNA molecule according to the process of claim 1,
- (b) combining said first and said complement cDNA strands in a reaction mixture with first primers and second primers, each of which contains a non-replicable element and/or a cleavable element, under conditions such that a first generation primer extension product is synthesized using said strands as templates, and wherein the first and second primers are selected such that the first generation primer extension products, when separated from their

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templates, can serve as templates for synthesis of second generation primer extension products of the first and second primers;

- (c) separating the first generation primer extension products from their respective templates to produce single-stranded molecules; and
- (d) treating the first generation primer extension products with the first and second primers under conditions such that second generation primer extension products are synthesized using the first generation primer extension products as templates, wherein the second-generation primer extension products contain at least a portion of the sequence of the nucleic acid sequence of said first generation primer extension product and no more than an insufficient portion of the binding site for said first and second primers for producing said first-generation primer extension products.

4. (Amended) A process for amplifying a cDNA molecule comprising:

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- (a) generating a cDNA molecule according to the process of claim 1 ;
- (b) combining said first and said complement cDNA strands in a reaction mixture with a series of nested primers, each nested primer containing a non-replicable element and/or a cleavable element, said series of nested primers comprising a plurality of primers which are complementary to different portions of said strands and are 5' to one another with respect to said strand and which do not overlap with one another at the position of said non-replicable element or cleavable element;
- (c) subjecting said reaction mixture to conditions such that first generation primer extension products are produced from first nested primers, and not from other of said series of nested primers using the first and second strands as templates, wherein said first nested primers are primers of, said nested primers which are most 3' with respect to said sequence of said first and second cDNA strands, and wherein the first nested primers are selected such that a first generation primer extension product,

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when separated from its template, can serve as a template for synthesis of a second generation extension product of the first nested primer for the complement strand;

- (d) separating the first generation primer extension products from their respective templates to produce single-stranded molecules;
- (e) exposing said reaction mixture to conditions such that second generation primer extension products are generated by said first nested primers using first generation primer extension products as templates, wherein the second generation primer extension products contain at least portion of the sequence of the nucleic acid sequence of said first generation primer extension product and no more than an insufficient portion of the binding site for said first nested primers for producing said first generation primer extension products;
- (f) separating the second generation primer extension products from their template to produce single stranded molecules;

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- (g) subjecting the reaction mixture of step (f) to reaction conditions such that next generation primer extension products are synthesized from another nested primer of said series of nested primers using second generation primer extension products as templates, and separating the thus produced next generation primer extension products from their templates to produce single-stranded molecules; and
- (h) repeating step (g) such that each repeat of step (g) comprises subjecting the reaction mixture to conditions such that next generation primer extension products are synthesized from a different nested primer of said series of nested primers using the next prior generation primer extension products as templates.

5. (Amended) A process for amplifying a cDNA molecule comprising:

- (a) generating a cDNA molecule according to the process of claim 1;
- (b) combining said first and said complement strands in a reaction mixture with a series of nested primers, each nested primer containing a non-replicable

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element and/or a cleavable element, said series of nested primers comprising a plurality of primers which are complementary to different portions of said strands and flank the sequence of interest but do not overlap with one another at the position of said non-replicable element or cleavable element;

- (c) subjecting said reaction mixture to conditions whereby each of said nested primers is capable of binding to its respective complementary site;
- (d) separating the first generation primer extension products from their respective templates to produce single-stranded molecules; and
- (e) repeating steps (c) and (d) whereby next generation primer extension products are synthesized from a different nested primer of said series of nested primers using the next prior generation primer extension products as templates.

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8. (Amended) The process of anyone of claims 2-5, wherein said primer of step (b) of the dependent claim contains a non-replicable element.

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9. (Amended) The process of anyone of claims 2-5, wherein said primer of step (b) of the dependent claim contains a cleavable element.
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16. (Amended) A process for generating a complementary DNA (cDNA) molecule from an RNA molecule comprising:
- a) annealing a primer containing a non-replicable element, with or without a cleavable element, to an RNA molecule, and
 - b) generating a first strand cDNA product.
17. (Amended) A process for amplifying a cDNA molecule comprising:
- a) generating a cDNA molecule according to the process of claim 16;
 - b) combining the cDNA strand in a reaction mixture with a primer containing a non-replicable element and/or a cleavable element, under conditions such that first-generation primer extension product is produced using said strand as a template, and wherein the primer for said strand is selected such that a first generation primer extension product produced using said strand as a template, when separated from said strand, can serve as

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a template for synthesis of a second generation primer extension product of a primer for the first generation primer extension product;

- c) separating the first generation primer extension products from their respective templates to produce single-stranded molecules; and
- d) treating the first generation primer extension products with the primers described in step (b) under conditions such that second generation primer extension products are produced using the first generation primer extension products as templates, wherein the second generation primer extension products contain at least a portion of the sequence of the nucleic acid sequence of the first generation primer extension product and no more than an insufficient portion of the binding site for said first primers for producing said first generation primer extension products.

18. (Amended) A process for amplifying a cDNA molecule comprising:

- a) generating a cDNA molecule according to the process of claim 16;

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- b) combining the first and second cDNA strands in a reaction mixture with first primers and second primers, each of said first and second primers containing a non-replicable element and/or a cleavable element, under conditions such that a first generation primer extension product is synthesized using said strands as templates, and wherein the first and second primers are selected such that the first generation primer extension products, when separated from their templates, can serve as templates for synthesis of second generation primer extension products of the first and second primers;
- c) separating the first generation primer extension products from their respective templates to produce single-stranded molecules; and
- d) treating the first generation primer extension products with the first and second primers under conditions such that second generation primer extension products are synthesized using the first generation primer extension products as templates, wherein the second generation primer extension products contain at least a portion of the sequence of the nucleic acid sequence of the first generation primer extension product and no more than an

B26
contd

insufficient portion of the binding site for said first and second primers for producing said first generation primer extension products.

19. (Amended) A process for amplifying a cDNA molecule comprising:

- a) generating a cDNA molecule according to the process of claim 16;
- b) combining the first and second cDNA strands in a reaction mixture with a series of nested primers, each nested primer containing a non-replicable element and/or a cleavable element, said series of nested primers comprising a plurality of primers which are complementary to different portions of said strands and are 5' to one another with respect to said strands and which do not overlap with one another at the position of said non-replicable element or cleavable element;
- c) subjecting said reaction mixture to conditions such that first generation primer extension products are produced from first nested primers, and not from other of said series of nested primers using the first and second strands as templates, wherein said first nested primers are primers of said nested primers which are most 3' with

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respect to said sequence, and wherein the first nested primers are selected such that a first generation primer extension product, when separated from its template, can serve as a template for synthesis of a second generation extension product of the first nested primer for the complement strand;

- d) separating the first generation primer extension products from their respective templates to produce single-stranded molecules;
- e) exposing said reaction mixture to conditions such that second generation primer extension products are generated by said first nested primers using first generation primer extension products as templates, wherein the second generation primer extension products contain at least a portion of the sequence of the nucleic acid sequence of the first generation primer extension product and no more than an insufficient portion of the binding site for said first nested primers for producing said first generation primer extension products;
- f) separating the second generation primer extension products from their template to produce single stranded molecules;

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control

- g) subjecting the reaction mixture of step (f) to reaction conditions such that next generation primer extension products are synthesized from another nested primer of said series of nested primers using second generation primer extension products as templates, and separating the thus produced next generation primer extension products from their templates to produce single-stranded molecules; and
- h) repeating step (g) such that each repeat of step (g) comprises subjecting the reaction mixture to conditions such that next generation primer extension products are synthesized from a different nested primer of said series of nested primers using the next prior generation primer extension products as templates.

20. (Amended) A process for amplifying a cDNA molecule comprising:

- a) generating a cDNA molecule according to the process of claims 16;
- b) combining the first and second strands in a reaction mixture with a series of nested primers, each nested primer containing a non-replicable element and/or a cleavable element, said series of nested primers

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contd

comprising a plurality of primers which are complementary to different portions of said strands and flank the sequence of interest but do not overlap with one another at the position of said non-replicable element or cleavable element;

- c) subjecting said reaction mixture to conditions whereby each of said nested primers is capable of binding to its respective complementary site;
- d) separating the first generation primer extension products from their respective templates to produce single-stranded molecules; and
- e) repeating steps (c) and (d) whereby next generation primer extension products are synthesized from a different nested primer of said series of nested primers using the next prior generation primer extension products as templates.

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- 23. (Amended) The process of anyone of claims 17 -20, wherein said primer of step (b) of the dependent claim contains a non-replicable element.
 - 24. (Amended) The process of any one of claims 17 -20, wherein said primer of step (b) of the dependent claim contains a cleavable element.
-

REMARKS

The Specification has been amended to insert SEQ ID NOs. The claims have been amended to more clearly describe the invention. No new matter has been added.

Objections to the Specification

The Examiner objects to the Specification because the sequences therein are not associated with SEQ ID NOs. In addition, the Examiner points out two typographical errors in the claims. Applicants have amended the Specification to insert the appropriate SEQ ID NO with the associated sequence and have amended the claims to correct the typographical errors.

Rejections under 35 USC 112, second paragraph

The Examiner has rejected claims 2-15 and 17-30 as indefinite. Specifically, the Examiner points to the use of the phrase "the sequence of the nucleic acid sequence of interest" and "the sequence of interest" in claims 2-15 and the use of the phrase "primers of step (b)" in the same claims. Applicants have amended the claims to replace these phrases with more definite language.

The Examiner has rejected claims 2 and 6-15 for use of the phrase "the primer for the first strand" and "said primers for producing said first generation primer extension product" contending a lack of proper antecedent basis. Applicants have amended these claims to clearly indicate the antecedent basis and to make the claims more clear.

The Examiner has rejected claims 3 and 6-15 for use of the phrase "said first and second primers containing a non-replicable element and/or a cleavable element." Applicants have amended the claims to clearly indicate the identity of these primers.

The Examiner has rejected claims 4, 6-15, 19 and 21-30 as lacking proper antecedent basis for the phrases "this step" and "said strand." Applicants have amended the claims to clearly indicate the antecedent basis.

The Examiner has rejected claims 4-15 as unclear because of the use of the phrases "each primer" and "said first primers are primers of said nested primers." Applicants have amended the claims as suggested by the Examiner and to clearly indicate the identity of the primers.

The Examiner has rejected claims 8, 9-11 and 23-26 as indefinite for the use of the phrase "said primer." Applicants have amended the claims to clearly indicate the identity of the primer.

The Examiner has rejected claim 17 as indefinite for the use of phrases "said strands" and "said primers of step (b)." Applicants have amended the claims to clearly indicate the identity of the strands and the primers.

The Examiner has rejected claims 17-30 as indefinite for the use of the phrase "the sequence of interest." Applicants have amended the claim, deleting this phrase.

The Examiner has rejected claims 19-30 as indefinite for the use of the phrase "each primer." Applicants have amended the claims to read "each nested primer."

The Examiner has rejected claims 19-30 as indefinite for the use of the phrase "said first primers are primers of said nested primers." Applicants have amended the claims to clearly indicate the identity of the primers.

Rejections under 35 USC § 102(e)

The Examiner has rejected claims 1-3, 6-13, 16-18 and 21-28 as being anticipated by Newton et al. (US Patent No. 5,525,494). The Examiner contends that Newton teaches amplification method using a first and second primer comprising a "non-replicable element." The Examiner also contends that Newton teaches "a method for amplifying a target nucleic acid wherein the method comprises

contacting a sample nucleic acid, together or sequentially, with a first primer comprising a target binding region and a polynucleotide tail and with an amplification primer comprising a target binding region and a polynucleotide tail, in the presence of a polymerization agent under conditions in which the first primer and amplification primer anneal to the target nucleic acid and wherein the first primer and the amplification primer are extended to generate an amplification product." Applicants respectfully traverse.

There is nothing in the Newton reference that discloses or suggests the claimed use of primers containing non-replicable elements or cleavable elements to halt primer extension products. Newton presents a PCR process which, like PCR with "normal" primers, is subject to the deficiencies noted in the instant Specification. Newton actually teaches away from the presently-claimed process. Newton includes the blocking agent in the template, rather than the primer, and thus produces single-stranded tails on amplification products which are used for solid phase capture (e.g. in microtiter dish assays). Newton also uses the primers in PCR reactions where molecules that would otherwise correspond to Applicant's second generation primer extension products are functioning as templates in subsequent rounds of

primer extension using the same primer set. Nothing in Newton suggests stopping primer extension after the generation of only second generation primer extension products, which is a feature asserted in the instant claims. Consequently, Applicants respectfully request reconsideration and removal of the rejection.

In view of the above remarks, all the claims remaining in the case are submitted as defining non-obvious, patentable subject matter. Reconsideration of the rejections and allowance of the claims are respectfully requested.

If the Examiner has any questions concerning this application, the Examiner is requested to contact the undersigned at (714) 708-8555 in California.

If necessary, the Commissioner is hereby authorized in this, concurrent and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

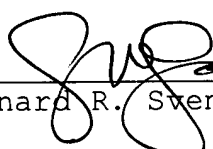
Pursuant to the provisions of 37 C.F.R. §§ 1.17 and 1.136(a), the Applicant petition for an extension of three months to April 3, 2002 for the period in which to file a response to the Office Action dated October 3, 2001.

Appl. No. 09/544,773
Response filed on April 7, 2000

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. §§ 1.16 or 1.17; particularly, extension of time fees.

Respectfully submitted,

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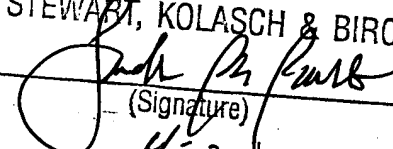
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(Date of deposit)

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(Signature)
4-3-02
(Date of Signature)